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=> Thymidine (w) kinase or TK
         56289 THYMIDINE
           340 THYMIDINES
         56413 THYMIDINE
                 (THYMIDINE OR THYMIDINES)
        331071 KINASE
         62323 KINASES
        341235 KINASE
                 (KINASE OR KINASES)
         10345 THYMIDINE (W) KINASE
         11562 TK
           433 TKS
         11810 TK
                 (TK OR TKS)
L1       17808 THYMIDINE (W) KINASE OR TK

=> vaccinia
         11608 VACCINIA
             2 VACCINIAS
L2       11609 VACCINIA
                 (VACCINIA OR VACCINIAS)

=> L1 (p) L2
L3         508 L1 (P) L2

=> point (w) mutation
        739852 POINT
        228591 POINTS
        921878 POINT
```

```
                (POINT OR POINTS)
        282659 MUTATION
        185928 MUTATIONS
        352944 MUTATION
                (MUTATION OR MUTATIONS)
L4       25594 POINT (W) MUTATION

=> L4 (1) L3
L5           0 L4 (L) L3

=> L4 and L3
L6           0 L4 AND L3

=> TK (1) mutation
         11562 TK
           433 TKS
         11810 TK
                (TK OR TKS)
        282659 MUTATION
        185928 MUTATIONS
        352944 MUTATION
                (MUTATION OR MUTATIONS)
L7         919 TK (L) MUTATION

=> L7 and L4
L8         112 L7 AND L4

=> L3 and L8
L9           0 L3 AND L8

=> vaccinia (s) L8
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'VACCINIA (S) L8'
         11608 VACCINIA
             2 VACCINIAS
         11609 VACCINIA
                (VACCINIA OR VACCINIAS)
L10          0 VACCINIA (S) L8

=> vaccinia
         11608 VACCINIA
             2 VACCINIAS
L11      11609 VACCINIA
                (VACCINIA OR VACCINIAS)

=> L11 (1) L8
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L11 (L) L8'
L12          0 L11 (L) L8

=> copenhagen (1) vaccinia
          1742 COPENHAGEN
         11608 VACCINIA
             2 VACCINIAS
         11609 VACCINIA
                (VACCINIA OR VACCINIAS)
L13        111 COPENHAGEN (L) VACCINIA

=> L13 and L7
L14          1 L13 AND L7
```

```
=> L4 and TK
         11562 TK
           433 TKS
         11810 TK
                 (TK OR TKS)
L15        121 L4 AND TK

=> L4 (s) L2
L16         14 L4 (S) L2

=> D L16 IBIB ABS 1-14

L16  ANSWER 1 OF 14  CAPLUS  COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER:         2007:594241  CAPLUS
DOCUMENT NUMBER:          147:379056
TITLE:                    Microarray assay for evaluation of the genetic
                          stability of modified vaccinia virus Ankara B5R gene
AUTHOR(S):                Laassri, Majid; Meseda, Clement A.; Williams, Ollie;
                          Merchlinsky, Michael; Weir, Jerry P.; Chumakov,
                          Konstantin
CORPORATE SOURCE:         Center for Biologics Evaluation and Research, US Food
                          and Drug Administration, Rockville, MD, 20852, USA
SOURCE:                   Journal of Medical Virology (2007), 79(6), 791-802
                          CODEN: JMVIDB; ISSN: 0146-6615
PUBLISHER:                Wiley-Liss, Inc.
DOCUMENT TYPE:            Journal
LANGUAGE:                 English
AB   Adverse events associated with the use of live smallpox vaccines have led to
     the development of a new generation of attenuated smallpox vaccines that
     are prepared in cultured cells as alternatives.  The inability to conduct
     direct clin. evaluation of their efficacy in humans demands that licensure
     be based on animal studies and exhaustive evaluation of their in vitro
     properties.  One of the most important characteristics of live viral
     vaccines is their genetic stability, including reversion of the vaccine
     strain to more virulent forms, recombination with other viral sequences to
     produce potentially pathogenic viruses, and genetic drift that can result
     in decrease of immunogenicity and efficacy.  To study genetic stability of
     an immunoessential vaccinia virus gene in a new generation smallpox
     vaccine, an advanced oligonucleotide microchip was developed and used to
     assay for mutations that could emerge in B5R gene, a vaccinia virus gene
     encoding for a protein that contains very important neutralizing epitopes.
     This microarray contained overlapping oligonucleotides covering the B5R
     gene of modified vaccinia virus Ankara (MVA), a well-studied candidate
     smallpox vaccine.  The microarray assay was shown to be able to detect
     even a single point mutation, and to differentiate
     between vaccinia strains.  At the same time, it could detect
     newly emerged mutations in clones of vaccinia strains.  In the work
     described here, it was shown that MVA B5R gene was stable after 34
     passages in Vero and MRC-5 cells that were proposed for use as cell
     substrates for vaccine manufacture  Potentially, the proposed method could be
     used as an identity test and could be extended for the entire viral genome
     and used to monitor consistency of vaccine production
REFERENCE COUNT:          38    THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16  ANSWER 2 OF 14  CAPLUS  COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER:         2003:837254  CAPLUS
DOCUMENT NUMBER:          139:302989
TITLE:                    VAC-BAC shuttle vector system comprising modified
                          vaccinia virus and use for gene expression
INVENTOR(S):              Moss, Bernard; Domi, Arban
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PATENT ASSIGNEE(S): Government of the United States of America, as Represented by the Secretary Department of Health and Human Services, USA
SOURCE: PCT Int. Appl., 36 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003087330	A2	20031023	WO 2003-US11183	20030410
WO 2003087330	A3	20040325		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2003221885	A1	20031027	AU 2003-221885	20030410
EP 1495125	A2	20050112	EP 2003-718343	20030410
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 20050124070	A1	20050609	US 2004-959392	20041005
PRIORITY APPLN. INFO.:			US 2002-371840P	P 20020410
			US 2002-402824P	P 20020809
			WO 2003-US11183	W 20030410

AB The invention relates to a VAC-BAC shuttle vector system for creation of recombinant poxviruses from DNA cloned in a bacterial artificial chromosome. The VAC-BAC vector system contains a vaccinia virus genome (VAC) that can replicate in bacteria and produce infectious virus in mammalian cells. The VAC-BAC vector system can be used to modify vaccinia virus DNA by deletion, insertion or point mutation or add new DNA to the VAC genome with methods developed for bacterial plasmids, rather than by recombination in mammalian cells. It can also be used to produce recombinant vaccinia viruses for gene expression and production of modified vaccinia viruses that have improved safety or immunogenicity.

L16 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1998:113288 CAPLUS
DOCUMENT NUMBER: 128:239981
ORIGINAL REFERENCE NO.: 128:47397a,47400a
TITLE: PCR-based method for the introduction of mutations in genes cloned and expressed in vaccinia virus
AUTHOR(S): Lorenzo, Maria del Mar; Blasco, Rafael
CORPORATE SOURCE: Cent. Invest. Sanidad Animal, INIA, Madrid, Spain
SOURCE: BioTechniques (1998), 24(2), 308-313
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Vaccinia virus expression systems allow efficient expression of genes and facilitate functional studies of expressed proteins in cultured mammalian cells. We designed and tested a rapid method to introduce defined mutations in genes inserted and expressed in vaccinia virus. PCR

mutagenesis is used to construct a recombination cassette that contains: (i) the mutated exogenous gene, (ii) recombination flanks to direct insertion into the virus genome and (iii) a selectable gene to allow easy isolation of recombinant viruses. To generate recombinant viruses, the recombination cassette is transfected into vaccinia virus-infected cells. The procedure does not require cloning, and the mutated gene versions are inserted directly into the vaccinia virus genome downstream of a vaccinia virus strong promoter. The method was tested by introducing a point mutation into Aequorea victoria green fluorescent protein (GFP), known to alter the fluorescence absorption and emission spectra of the protein. This system should facilitate and speed the isolation of virus recombinants expressing mutated versions of any given gene and can be adapted to random mutagenesis procedures, exon shuffling or other PCR-based mutagenesis protocols.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1997:224524 CAPLUS

DOCUMENT NUMBER: 126:292107

ORIGINAL REFERENCE NO.: 126:56541a,56544a

TITLE: Point mutation flanking a CTL epitope ablates in vitro and in vivo recognition of a full-length viral protein

AUTHOR(S): Yellen-Shaw, Amy J.; Wherry, E. John; Dubois, Garrett C.; Eisenlohr, Laurence C.

CORPORATE SOURCE: Kimmel Cancer Inst., Jefferson Med. Coll., Philadelphia, PA, 19107, USA

SOURCE: Journal of Immunology (1997), 158(7), 3227-3234 CODEN: JOIMA3; ISSN: 0022-1767

PUBLISHER: American Association of Immunologists

DOCUMENT TYPE: Journal

LANGUAGE: English

AB CD8+ T cells (TCD8+) recognize viral Ags as short peptides (epitopes) displayed at the cell surface by MHC class I mols. Using a panel of recombinant vaccinia viruses, the authors show that single-point mutations flanking either side of an H-2Kd-restricted epitope, residues 147-155, within full-length influenza nucleoprotein (NP) can impact, even ablate, presentation of that epitope, while having no effect on presentation of distal epitopes. The most severe blocking mutation (Ala to Pro at position 146) did not inhibit NP147-155 presentation in the context of a truncated minigene, implying that this peptide is not a functional processing intermediate. An N-terminal proline replacement also reduced presentation of NP50-57 (H-2Kk restricted), while the same mutation did not affect a third NP epitope. Thus, while trends in processing specificity may exist, the epitope itself contributes to flanking sequence effects. These findings were paralleled by in vivo priming expts. in which, depending on viral dose, subtle in vitro blocking effects were absolute Proteasome/synthetic peptide coincubation studies support a role for enhanced epitope destruction in preventing presentation, as did the effect of the peptide aldehyde, LLnL, which restored presentation of NP147-155 from the mutated constructs. This reagent did not inhibit epitope presentation, even from wild-type NP, suggesting that its production may be proteasome independent. These results support the notion that point mutation of epitope flanking sequence can serve as a mechanism for viral immune evasion, shed light on the mechanisms involved, and suggest that in vitro assays may not be sensitive indicators of flanking sequence effects.

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 1996:33930 CAPLUS
DOCUMENT NUMBER: 124:79991
ORIGINAL REFERENCE NO.: 124:14845a,14848a
TITLE: Definition and functional analysis of the signal/anchor domain of the human respiratory syncytial virus glycoprotein G
AUTHOR(S): Lichtenstein, Drew L.; Roberts, Sharon R.; Wertz, Gail W.; Ball, L. Andrew
CORPORATE SOURCE: Dep. Biochem., Univ. Wisconsin-Madison, Madison, WI, 53706, USA
SOURCE: Journal of General Virology (1996), 77(1), 109-18
CODEN: JGVIAY; ISSN: 0022-1317
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The attachment protein G of human respiratory syncytial (RS) virus is a type II transmembrane glycoprotein. A secreted form of the G protein is also produced. To examine the two distinct hydrophobic regions in the N-terminal 63 amino acids of G protein for their role(s) in membrane insertion and anchoring, transport to the cell surface, and secretion, G proteins that contained point mutations or deletions were synthesized by cell-free transcription-translation and in cells by expression from recombinant vaccinia virus vectors. A mutant protein lacking the entire major hydrophobic region (amino acids 38-63) was not glycosylated, not expressed on the cell surface, and not secreted, because it was not inserted into membranes. In contrast, deletion of the minor hydrophobic region (amino acids 23-31) had no detectable effect on membrane insertion or anchoring. These data provided direct evidence that amino acids 38-63 were necessary for membrane insertion and contained the signal/anchor domain of RS virus G protein. Mutant proteins that lacked either the N-terminal or the C-terminal half of this 26 residue hydrophobic region were inserted into membranes and processed to maturity, showing that either half of this region was sufficient for membrane insertion. However, these two mutant proteins were secreted more abundantly than wild-type G protein. We propose that their truncated hydrophobic domains interacted with membranes in a way that mimicked the N-terminal signal sequence of naturally secreted proteins, allowing proteolytic cleavage of the mutant proteins.

L16 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1994:129324 CAPLUS
DOCUMENT NUMBER: 120:129324
ORIGINAL REFERENCE NO.: 120:22681a,22684a
TITLE: Assembly of vaccinia virus: effects of rifampin on the intracellular distribution of viral protein p65
AUTHOR(S): Sodeik, Beate; Griffiths, Gareth; Ericsson, Maria; Moss, Bernard; Doms, Robert W.
CORPORATE SOURCE: Cell Biol. Program, Eur. Mol. Biol. Lab., Heidelberg, 69012, Germany
SOURCE: Journal of Virology (1994), 68(2), 1103-14
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The cytoplasmic assembly of vaccinia virus is reversibly blocked by the antibiotic rifampin, leading to the accumulation of partially membrane-delineated rifampin bodies in infected cells. Rifampin-resistant vaccinia virus mutants have point mutations in the D13L gene, which is controlled by a late promoter and expresses a 65-kDa protein, designating p65. To further characterize the mechanism of rifampin inhibition and the function of p65 in virus assembly, the authors raised antibodies to this protein. Immunoreactive p65 was expressed at

late times of infection, and neither its expression nor its turnover were affected by rifampin. Virus-associated p65 could be extracted only with denaturing detergents from purified virions, suggesting that it is an integral viral component. Immunofluorescence studies showed that p65 is localized to the sites of virus assembly. Also, immunoelectron microscopy showed p65 to be associated with viral crescents as well as spherical, immature virions, in both cases predominantly on the inner or concave surface. In the presence of rifampin, p65 was found in large, cytoplasmic inclusion bodies that were distinct from rifampin bodies. The rifampin bodies themselves were labeled with p65 antibodies only after reversal of the rifampin block, predominantly on the viral crescents which rapidly formed following removal of the drug. The authors propose that p65 functions as an internal scaffold in the formation of viral crescents and immature virions, analogously to the matrix proteins of other viruses.

L16 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1994:100495 CAPLUS
DOCUMENT NUMBER: 120:100495
ORIGINAL REFERENCE NO.: 120:17675a,17678a
TITLE: Genetic characterization of the vaccinia virus DNA polymerase: identification of point mutations conferring drug-resistance and altered fidelity
AUTHOR(S): Taddie, John Anthony
CORPORATE SOURCE: Med. Coll., Cornell Univ., New York, NY, USA
SOURCE: (1992) 234 pp. Avail.: Univ. Microfilms Int., Order No. DA9233448
From: Diss. Abstr. Int. B 1992, 53(6), 2699
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L16 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1993:663795 CAPLUS
DOCUMENT NUMBER: 119:263795
ORIGINAL REFERENCE NO.: 119:47005a,47008a
TITLE: Cloning of Chinese hamster DNA topoisomerase I cDNA and identification of a single point mutation responsible for camptothecin resistance
AUTHOR(S): Tanizawa, Akihiko; Bertrand, Richard; Kohlhagen, Glenda; Tabuchi, Arata; Jenkins, Jeffrey; Pommier, Yves
CORPORATE SOURCE: Lab. Mol. Pharmacol., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Journal of Biological Chemistry (1993), 268(34), 25463-8
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A camptothecin-resistant (DC3F/C-10) Chinese hamster cell line that contains a catalytically altered and camptothecin (CPT)-resistant DNA topoisomerase I (top 1) (Tanizawa, A., and Pommier, Y., 1992) and the parent cell line (DC3F) were used to compare top 1 mRNAs and cDNAs. Northern blot anal. showed a single 4.1-kilobase band without quant. reduction between the two cell lines. The authors have cloned and sequenced top 1 cDNAs. DC3F and DC3F/C-10 top 1 c-DNA are 3591 and 3626 base pair long, resp., and encode 767 amino acids. The homol. of deduced amino acid sequences between Chinese hamster and mouse or human top 1 are 98.1 and 96.7, resp. CDNAs from DC3F/C-10 and DC3F cells differ by a single base point mutation (G to A) which results in an amino acid change from Gly505 to Ser (Gly505 → Ser). G505 corresponds to Gly503 of human top 1

cDNA and is located 220 amino acids away from the presumed catalytic Tyr725. The point mutation in the Chinese hamster top 1 is located in a region that is highly conserved among all cloned top 1 cDNAs (plant ATH, vaccinia virus, Shope fibroma virus, Drosophila, Saccharomyces cerevisiae, Schizosaccharomyces pombe, mouse, and Human). A mutation of Asp533 to Gly in this same region has been shown to confer CPT resistance for human top 1. Chinese hamster top 1 protein with a Gly505 → Ser mutation that was expressed in bacteria was resistant to CPT, indicating that this single base mutation is involved in CPT resistance. The data suggest that the highly conserved region around Gly505 plays an important role in the interactions among top 1, DNA, and CPT.

L16 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1993:575230 CAPLUS
DOCUMENT NUMBER: 119:175230
ORIGINAL REFERENCE NO.: 119:31171a,31174a
TITLE: Dissociation of progeny vaccinia virus from the cell membrane is regulated by a viral envelope glycoprotein: Effect of a point mutation in the lectin homology domain of the A34R gene
AUTHOR(S): Blasco, Rafael; Sisler, Jerry R.; Moss, Bernard
CORPORATE SOURCE: Lab. Viral Dis., Natl. Inst. Allergy and Infect. Dis., Bethesda, MD, 20892, USA
SOURCE: Journal of Virology (1993), 67(6), 3319-25
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Vaccinia virus strains vary considerably in the amts. of extracellular enveloped virus (EEV) that they release from infected cells. The IHD-J strain produces ≤40 times more EEV than does the related WR strain and consequently generates elongated comet-shaped virus plaques instead of sharply defined round ones in susceptible monolayer cells under liquid medium. The difference of EEV formation is due to the retention of enveloped WR virions on the cell surface. By using WR and IHD-J DNA fragments for marker transfer and analyzing the progeny virus by the comet formation assay, the authors determined that gene A34R and at least one other gene regulate the release of cell-associated virions. Replacement of the A34R gene of WR with the corresponding gene from IHD-J increased the amount of EEV produced by 10-fold and conferred the ability to form distinctive comet-shaped plaques. Gene A34R encodes on EEV-specific glycoprotein with homol. to c-type animal lectins. The nucleotide sequences of the A34R genes of WR and IHD-J strains differed in 6 positions, of which 4 were silent. One of the codon mutations (Lys-151→Glu), which is located in the putative carbohydrate recognition domain, was sufficient to transfer a comet-forming phenotype to WR virus. These data indicate that the A34R-encoded glycoprotein is involved, through its lectin homol. domain, in the retention of progeny virus on the surface of parental cells and raise the possibility that the protein also has a role in virus attachment to uninfected cells.

L16 ANSWER 10 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1991:507327 CAPLUS
DOCUMENT NUMBER: 115:107327
ORIGINAL REFERENCE NO.: 115:18257a,18260a
TITLE: A temperature-sensitive lesion in the small subunit of the vaccinia virus-encoded mRNA capping enzyme causes a defect in viral telomere resolution
AUTHOR(S): Carpenter, Michael S.; DeLange, Aloysius M.
CORPORATE SOURCE: Dep. Hum. Genet., Univ. Manitoba, Winnipeg, MB, R3E

0W3, Can.
SOURCE: Journal of Virology (1991), 65(8), 4042-50
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The temperature-sensitive (ts) conditional lethal mutant ts9383 was shown to be,
at the nonpermissive temperature, defective in the resolution of concatemeric replicative intermediate DNA to linear 185-kb monomeric DNA genomes. The resolution defect was the result of a partial failure of the mutant virus to convert the replicated form of the viral telomere to hairpin termini. In contrast to other mutants of this phenotype, pulse-labeling of viral proteins at various times postinfection revealed no obvious difference in the quantity or temporal appearance of members of the late class of polypeptides. Using the marker rescue technique, the ts lesion in ts9383 was localized to an approx. 1-kb region within the HindIIID fragment. Both the ts phenotype and the resolution defect were caused by a single-base C→T point mutation resulting in the conversion of the amino acid proline to serine in codon 23 of open reading frame D12. This gene encodes a 33-kDa polypeptide which is known to be the small subunit of the virus-encoded mRNA capping enzyme. The data are consistent with a role for this capping enzyme subunit during poxviral telomere resolution

L16 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1991:442834 CAPLUS
DOCUMENT NUMBER: 115:42834
ORIGINAL REFERENCE NO.: 115:7305a,7308a
TITLE: Genetic characterization of the vaccinia virus DNA polymerase: identification of point mutations conferring altered drug sensitivities and reduced fidelity
AUTHOR(S): Taddie, John A.; Traktman, Paula
CORPORATE SOURCE: Grad. Sch. Med. Sci., Cornell Univ., New York, NY, 10021, USA
SOURCE: Journal of Virology (1991), 65(2), 869-79
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB It was determined that 85 μM aphidicolin was sufficient to block macroscopic plaque formation by vaccinia virus and to cause a 104-fold reduction in viral yield from a wild-type infection. A chemical mutagenized viral stock was passaged sequentially in the presence of drug, and plaque-purified viral stocks resistant to aphidicolin were isolated and characterized. By use of a marker rescue protocol, the lesion in each mutant was found to map within the same 500-bp fragment within the DNA polymerase gene. All of the mutants were found to contain a single nucleotide change in the same codon. In 9 of these mutants, the alanine residue at position 498 was changed to a threonine, whereas a 10th mutant sustained a valine substitution at this position. Congenic viral strains which carried the Aphr lesion in an unmutagenized wild-type background were isolated. The Thr and Val mutations were found to confer equivalent levels of drug resistance. In the presence of drug, viral yields were 25% of control levels, and the levels of viral DNA synthesized were 30 to 50% of those seen in control infections. The 2 mutations also conferred an equivalent hypersensitivity to the cytosine analog 1-β-D-arabinofuranosylcytosine (araC); strains carrying the Thr mutation were moderately hypersensitive to the pyrophosphate analog phosphonoacetic acid and the adenosine analog araA, whereas the Val mutation conferred acute hypersensitivity to these inhibitors. The Val mutation also conferred a mutator phenotype, leading to a 20- to 40-fold increase in the frequency of spontaneous mutations within the viral stock.

L16 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1990:113148 CAPLUS
DOCUMENT NUMBER: 112:113148
ORIGINAL REFERENCE NO.: 112:19039a,19042a
TITLE: Identification of the point mutations in two vaccinia virus nucleoside triphosphate phosphohydrolase I temperature-sensitive mutants and role of this DNA-dependent ATPase enzyme in virus gene expression
AUTHOR(S): Kahn, Jeffrey S.; Esteban, Mariano
CORPORATE SOURCE: Health Sci. Cent., State Univ. New York, Brooklyn, NY, 11203, USA
SOURCE: Virology (1990), 174(2), 459-71
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The biol. function of the nucleoside triphosphate phosphohydrolase I (NTPase I) enzyme of vaccinia virus is not yet known. In this investigation, the genetic lesion of two temperature-sensitive mutants of vaccinia virus, ts50 and ts36, were identified as single point mutations contained within the 5' 615 nucleotides of the NTPase I gene (ts50, G to A at position 131; ts36, C to T at position 556). The point mutations result in amino acid substitutions of Gly to Glu-44 (ts50) and Pro to Ser-186 (ts36). In monkey BSC-40 cells, ts50 and ts36 behave phenotypically like wild-type virus with respect to replication and synthesis of viral DNA but are defective in late polypeptide synthesis. However, these two ts mutants displayed a drastically different phenotype in virus-infected human HeLa cells at the restrictive temperature; viral DNA replication did not occur, and late polypeptide synthesis was absent. Moreover, if the early block was overcome by a temperature shift-up, then HeLa cells infected with the ts mutants displayed a profile characteristic of defective late viral polypeptide synthesis. The results reveal that vaccinia NTPase I enzyme functions early and late in the viral replication cycle and that the phenotype of these ts mutants is dependent upon the cell type.

L16 ANSWER 13 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1990:4441 CAPLUS
DOCUMENT NUMBER: 112:4441
ORIGINAL REFERENCE NO.: 112:887a,890a
TITLE: Humoral immune response elicited by highly attenuated variants of vaccinia virus and by an attenuated recombinant expressing HIV-1 envelope protein
AUTHOR(S): Dallo, Shatha; Maa, Juehn Shin; Rodriguez, Juan Ramon; Rodriguez, Dolores; Esteban, Mariano
CORPORATE SOURCE: Health Sci. Cent., State Univ. New York, Brooklyn, NY, 11203, USA
SOURCE: Virology (1989), 173(1), 323-4
CODEN: VIRLAX; ISSN: 0042-6822
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Attenuated variants of vaccinia virus have excellent potential for the construction of safe recombinant live vaccines. Highly attenuated variants of vaccinia virus with several genetic markers and a variant recombinant were tested in Balb/c mice for their ability to induce humoral immune response. Mice primed with variants that had an 8-MDa deletion at the left end of the viral genome induced similar levels of circulating anti-vaccinia antibodies as the wild-type virus. However, mice primed with variants that had several genetic lesions (deletions and

point mutations) induced lower levels of circulating anti-vaccinia antibodies. Mice primed and boosted with a recombinant variant with several genetic lesions, and containing the complete envelope gene of the human immunodeficiency virus (HIV) and the bacterial β-galactosidase (β-gal) gene, induced significant antibody response to gp160 and β-gal. The antibody response to gp160 was markedly increased by successive inoculations with the recombinant variant. These findings provide evidence that the extent of activation of the immune system by vaccinia variants can be modulated by the nature of the virus genetic lesion. In addition, when these variants are used as recombinant vaccines, it is possible to induce low levels of circulating anti-vaccinia antibodies after priming and yet achieve significant antibody response to virus-expressed foreign antigens, even after repeated boosters. Such variants could be useful in the design of live recombinant viruses as safe vaccines.

L16 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1989:626143 CAPLUS
DOCUMENT NUMBER: 111:226143
ORIGINAL REFERENCE NO.: 111:37421a,37424a
TITLE: A single point mutation of Ala-25 to Asp in the 14,000-Mr envelope protein of vaccinia virus induces a size change that leads to the small plaque phenotype of the virus
AUTHOR(S): Gong, Shiaoching; Lai, Chingfeng; Dallo, Shatha; Esteban, Mariano
CORPORATE SOURCE: Health Sci. Cent., State Univ. New York, Brooklyn, NY, 11203-2098, USA
SOURCE: Journal of Virology (1989), 63(11), 4507-14
CODEN: JOVIAM; ISSN: 0022-538X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The mol. defect responsible for a structural and functional abnormality of the 14,000-mol.-weight (14K) envelope protein of vaccinia virus was identified. Through DNA sequence anal. of the entire 14K gene from wild-type vaccinia virus and 3 vaccinia virus mutants, a single base change of C to A was found that resulted in the substitution of Asp for Ala-25. This mutation is responsible for protein size abnormality, as documented by cell-free translation in a rabbit reticulocyte lysate of in vitro mRNA transcripts. In addition, marker rescue expts. showed that this mutation is responsible for the small plaque size phenotype of vaccinia virus mutants. The structural consequence of the point mutation is a possible turn in an alpha-helix domain with destabilization of a hydrophobic interaction at the N terminus, resulting in monomers and trimers of vaccinia virus 14K protein with decreased electrophoretic mobilities. The functional consequence of the point mutation is a reduction in virulence of the virus.

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L14 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
ACCESSION NUMBER: 1984:62620 CAPLUS
DOCUMENT NUMBER: 100:62620
ORIGINAL REFERENCE NO.: 100:9465a,9468a
TITLE: Mapping of a mutation site in the thymidine kinase gene of vaccinia virus by marker rescue
AUTHOR(S): Vassef, A.; Ben-Hamida, F.; Beaud, G.
CORPORATE SOURCE: Inst. Jacques Monod, Univ. Paris, Paris, 75251, Fr.
SOURCE: Annales de Virologie (1983), 134E(3), 375-85
CODEN: ANVIDL; ISSN: 0242-5017

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The thymidine kinase (TK) [9002-06-6]-deficient mutant 1004B of vaccinia virus derived from the IHD strain was rescued with intact genomic DNA obtained from the wild-type IHD or the Copenhagen strain of the virus. The mutant was also rescued by a plasmid (pBR-J) composed of pBR322 and the HindIII-J genomic fragment (cloned from the WR strain) which contains the wild-type TK gene. Plasmid pBR-J was cleaved with 8 restriction enzymes, some of which also cleaved the pBR322 portion of the plasmid, and the digestion products were used to rescue the TK- virus mutant. Cleaving pBR-J with EcoRI reduced the amount of rescued virus by 92%. Furthermore, the plot of the yields of rescued virus as a function of the position of enzyme cleavage sites revealed a minimal value and mapped a zero rescue value at .apprx.0.6 kilobase pair (kbp). This mapping of a mutation site in the 1004B TK - mutant was relatively precise (within 0.35 kbp), with no mapping of corresponding mRNA. Moreover, the length of the DNA involved in each recombination event for marker rescue with this cloned DNA did not exceed .apprx.2 kbp.

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